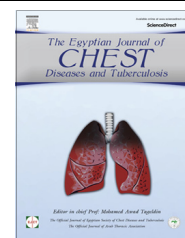




The Egyptian Society of Chest Diseases and Tuberculosis  
**Egyptian Journal of Chest Diseases and Tuberculosis**

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ORIGINAL ARTICLE

# Serum endocan role in diagnosis and prognosis of ventilator associated pneumonia



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Received 10 March 2015; accepted 21 May 2015

Available online 6 June 2015

## KEYWORDS

VAP;  
Endocan;  
APACHE II;  
SOFA;  
CPIS

**Abstract** Ventilator-associated pneumonia (VAP) is a complication in as many as 28% of patients who receive mechanical ventilation. Studies have consistently shown that a delay in diagnosis and treatment increases the mortality risk.

*Aim of the work:* The aim of this work is to elucidate the role of the serum endocan in the diagnosis and the prognosis of ventilator associated pneumonia.

*Methods:* Forty-two VAP patients, 20 non VAP ICU (on mechanical ventilation) admitted patients and 20 healthy control subjects of similar age and sex were included in the study. Endocan levels in serum samples were measured in all subjects.

*Results:* There was a highly statistically significant difference ( $p$  value  $<0.001$ ) between VAP patients on one side and non VAP-ICU patients and healthy control subjects on the other side regarding the mean values of endocan. Also, the mean values of endocan were statistically significantly higher ( $p < 0.001$ ) among the dead VAP group than the survivor VAP group. There was a high statistically positive correlation ( $p$  value  $<0.001$ ) between mortality prediction scores (APACHE II ( $R = 0.549$ ), CRIP ( $R = 0.599$ ) and SOFA ( $R = 0.517$ )) and endocan serum levels.

*Conclusions:* This study found increased endocan serum levels among VAP patients. We suggest a role for endocan in the diagnosis and prediction of the prognosis of VAP patients.

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## Introduction

Ventilator-associated pneumonia (VAP) is pneumonia that develops 48 h or longer after mechanical ventilation and is given by means of an endotracheal tube or tracheostomy. Ventilator-associated pneumonia (VAP) results from the

invasion of the lower respiratory tract and lung parenchyma by microorganisms. Intubation compromises the integrity of the oropharynx and trachea and allows oral and gastric secretions to enter the lower airways [1].

Ventilator associated pneumonia (VAP) is a complication in as many as 28% of patients who receive mechanical ventilation. The incidence of VAP increases with the duration of mechanical ventilation. Estimated rates are 3% per day for the first 5 days, 2% per day for days 6–10, and 1% per day after day 10 [2].

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Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.

<http://dx.doi.org/10.1016/j.ejcdt.2015.05.015>

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The crude mortality rate of VAP is 27–76%. Studies have consistently shown that a delay in starting appropriate and adequately dosed antibiotic therapy increases the mortality risk. One of the causes of the high mortality rate is the diagnostic delay [1,3,4].

Unlike community-acquired pneumonia, it may be difficult to determine whether pneumonia has developed in a hospitalized ventilator-dependent patient. Symptoms and signs usually are not conclusive. Although the plain chest roentgenogram remains an important component in the evaluation of hospitalized patients with suspected pneumonia, it is most helpful when it is normal and rules out pneumonia. Microscopy evaluation and culture of tracheal secretions and/or expectorated sputum are also frequently inconclusive for patients clinically suspected of having pneumonia, because the upper respiratory tract of most patients in the ICU is colonized with potential pulmonary pathogens, whether or not parenchymal pulmonary infection is present [5,6].

Endocan is a novel endothelium derived soluble dermatan sulfate proteoglycan (PG) [7,8]. It is encoded by the ESM1 gene [9,10]. This gene is mainly expressed in the endothelial cells in human lung and kidney tissues. The expression of this gene is regulated by cytokines, suggesting that it may play a role in endothelium dependent pathological disorders [11].

Sepsis and inflammation have associated endothelial dysfunction ranging from vasodilation, edema to coagulopathy, ischemia, and organ failure. Since inflammatory mediators (IL-1, TNF- $\alpha$ ) induce endocan expression, blood levels of this soluble PG may closely reflect the presence and severity of inflammation as well as the response to therapy [10]. De Freitas Caires et al. have recently described elevated blood levels of cathepsin G-cleaved endocan in patients with sepsis. This 14 kDa circulating protein (p14) is the fragment of endocan specifically cleaved by cathepsin G, a neutrophil-derived serine protease [12]. In another study, Scherpereel et al. observed that circulating endocan levels in blood was related to the severity of sepsis and also reflected the outcome of the patients [13].

#### *Aim of the work*

The aim of this work is to elucidate the role of the serum endocan in the diagnosis and the prognosis of ventilator associated pneumonia.

#### **Subjects and methods**

This study was conducted at the ICU of our university hospitals. Consent was taken from each included subject. The study included 3 groups of subjects (see Table 1):

*Group 1:* It included 42 VAP patients that were admitted to the ICU. The mean age was  $58.09 \pm 13.07$ . There were 27 (64.28%) males and 15 (35.72%) females.

*Group 2:* It included 20 non VAP ICU admitted patients (on mechanical ventilation). The mean age was  $56.90 \pm 10.54$ . There were 12 (60%) males and 8 (40%) females.

*Group 3:* It included 20 healthy control subjects. The mean age was  $53.85 \pm 11.26$ . There were 13 (65%) males and 7 (35%) females.

The three groups were matched for age and sex ( $p$  value  $> 0.05$ ).

All subjects included in this study underwent the following:

1. Complete history taking and physical examination.
2. Chest X-ray examination.
3. Complete blood picture (CBC), C reactive protein (CRP), liver and kidney functions tests
4. Arterial blood gases analysis (ABGs).
5. Acute Physiology and Chronic Health Evaluation II (APACHE II) score was calculated for patient groups only.
6. The Sequential Organ Failure Assessment score (SOFA) was calculated for patient groups only.
7. The Clinical Pulmonary Infection Score (CPIS) was calculated for patient groups only
8. Measurement of endocan serum levels: This was done in all subjects included in this study. Blood samples were taken and the sera were separated and stored at  $-80^{\circ}\text{C}$  for further endocan analysis. Endocan levels were determined with the commercially available enzyme-linked immunosorbent assay (ELISA; LUNGINNOV Systems, Lille, France) [12]. For VAP patients endocan measurement was done on the first day of diagnosis (for comparing with other subject groups) and then repeated on the 7th day of diagnosis.

*Pneumonia* was classified as VAP if it occurred after 48 h of mechanical ventilation and was not incubated before the initiation of the mechanical ventilation. Early-onset VAP was defined as that occurring during the first 4 days on mechanical ventilation, whereas VAP develops thereafter was classified as late-onset [8]. A diagnosis of pneumonia was suspected when a new, persistent infiltrate, consolidation, cavitation or pleural effusion was seen on chest X-rays and at least two of the following were observed: a body temperature below  $36^{\circ}\text{C}$  or above  $38^{\circ}\text{C}$ ; a white blood cell count lower than  $4000/\text{mm}^3$  or higher than  $11,000/\text{mm}^3$ ; and macroscopically purulent tracheal aspirate. Tracheal aspirate was classified as purulent or nonpurulent after visual inspection. Tracheal aspirate for quantitative culture had been obtained before antimicrobial treatment was started. Tracheal aspirate cultures yielding  $\geq 10^5$  CFU/ml were considered positive. We calculated the clinical pulmonary infection score (CPIS). Patients were assumed to have VAP when the CPIS was  $> 6$ . Patients received a diagnosis of VAP only after other medical conditions to which the presenting symptoms, signs or radiological findings could be attributed had been ruled out. For all patients in whom the clinical suspicion of VAP was confirmed, empirical antimicrobial therapy was started immediately and modified after culture results if needed. Patients were followed until resolution of pneumonia or death [9].

#### *Exclusion criteria*

Any patient that had any one of the following was excluded from this study: (1) Age below 18 years; (2) The development of pneumonia within 48 h of the beginning of mechanical ventilation; (3) Evidence of bacterial infection elsewhere; (4) Immuno-suppression; (5) Expected early death.

### Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences, version 16 for Windows (SPSS Inc., Chicago, IL, USA). Values of  $p < 0.05$  were considered statistically significant.

### Results

The total duration of mechanical ventilation among patients with VAP was  $15.35 \pm 9.21$  days compared with  $10.67 \pm 8.52$  days in patients without VAP ( $p$  value  $< 0.01$ ).

There were 15 (35.71%) early onset VAP patients and 27 (64.29%) late onset VAP patients.

The causative microbes among VAP patients are shown in Table 2.

There was highly statistically significant difference ( $p$  value  $< 0.001$ ) between VAP patients ( $11.37 \pm 2.53$  ng/ml) on one side and non VAP-ICU ( $5.07 \pm 3.50$  ng/ml) patients and healthy control subjects ( $4.12 \pm 2.9$  ng/ml) on the other side regarding the mean values of endocan (Table 3 and Fig. 1). No statistically significant difference ( $p$  value  $> 0.05$ )

**Table 1** Some characteristic data for the subjects included in this study.

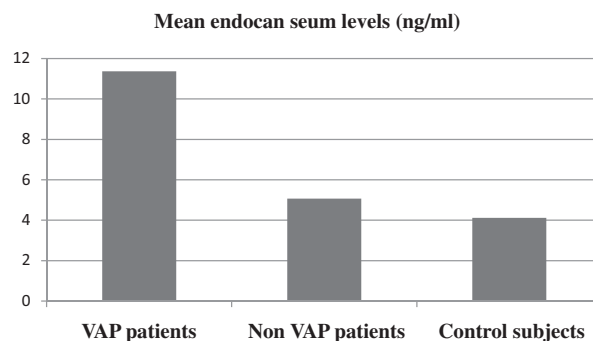
	VAP patients ( $N = 42$ )	Non VAP patients ( $N = 20$ )	Healthy subjects ( $N = 20$ )
Age (Mean $\pm$ SD)	$58.09 \pm 13.07$	$56.90 \pm 10.54$	$53.85 \pm 11.26$
Sex (Male/ Female)	27/15	12/8	13/7
CRP (mg/L)	$160.4 \pm 4.7$	$28.53 \pm 2.6$	$5.38 \pm 1.1$
WBC ( $\times 10^3/\mu\text{L}$ )	$14.5 \pm 3.53$	$9.6 \pm 0.45$	$6.5 \pm 1.04$
APACHE II	$24.11 \pm 4.57$	$16 \pm 3.25$	NA
SOFA	$7.88 \pm 2.92$	$3.56 \pm 2.04$	NA
<i>Associated conditions</i>			
COPD	8	5	0
DM	10	4	0
Renal failure	3	2	0
Heart diseases	11	5	0
Malignancy	7	2	0
Neurologic diseases	3	1	0
Liver cirrhosis	3	2	0

**Table 2** Microbiological profile among the VAP patients.

Microbes	No. of the VAP patients (%)
<i>Staphylococcus aureus</i>	7 (16.67)
<i>Pseudomonas aeruginosa</i>	5 (11.91)
<i>Klebsiella pneumoniae</i>	4 (9.52)
<i>Haemophilus influenzae</i>	3 (7.14)
<i>Acinetobacter</i>	2 (4.76)
<i>Enterobacter</i>	1 (2.38)
<i>Proteus</i>	1 (2.38)
<i>Serratia</i>	1 (2.38)
Mixed	2 (4.76)
Unidentified	16 (38.10)

**Table 3** Comparison between different subject groups regarding initial endocan levels (ng/ml).

Groups	Mean $\pm$ SD (ng/ml)	$p$ value
1. VAP patients	$11.37 \pm 2.53$	1 vs. 2 $< 0.01$
2. Non VAP patients	$5.07 \pm 3.50$	1 vs. 3 $< 0.01$
3. Control subjects	$4.12 \pm 2.9$	2 vs. 3 $> 0.05$



**Figure 1** Comparison between different subject groups regarding initial endocan levels (ng/ml).

in the mean values of endocan was present between non VAP-ICU patients ( $5.07 \pm 3.50$  ng/ml) and healthy control subjects ( $4.12 \pm 2.9$  ng/ml) (Table 3 and Fig. 1).

22 (52.38%) patients out of 42 VAP patients survived while 20 (47.62) patients died. No patient died in the first 7 days after diagnosis.

The mean values of endocan were statistically significantly higher ( $p$  value  $< 0.001$ ) among the dead VAP group than the survivor VAP group on both first day ( $12.50 \pm 2.74$  ng/ml vs.  $10.35 \pm 1.86$  ng/ml, respectively) and 7th day measurements ( $24.33 \pm 7.14$  ng/ml vs.  $5.93 \pm 1.56$  ng/ml respectively) (Table 4 and Fig. 2).

The mean values of endocan increased significantly (from  $12.50 \pm 2.74$  ng/ml to  $24.33 \pm 7.14$  ng/ml) among dead group while it decreased significantly (from  $12.50 \pm 2.74$  ng/ml to  $5.93 \pm 1.56$  ng/ml) among the survivor group on repeated testing on the 7th day in comparison with the first day measurement (Table 4 and Fig. 2).

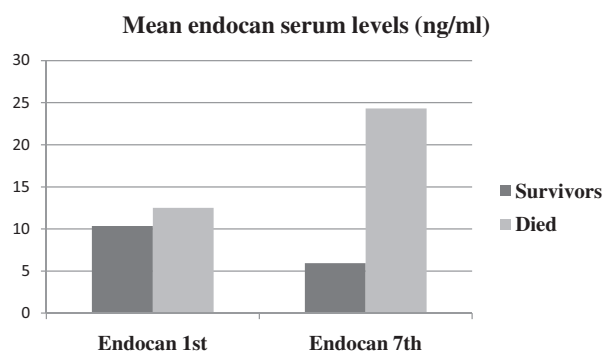
There was a high statistically positive correlation ( $p$  value  $< 0.001$ ) between mortality prediction scores (APACHE II ( $R = 0.549$ ), CRIP ( $R = 0.599$ ) and SOFA ( $R = 0.517$ )) and endocan serum levels.

### Discussion

In this study, there was a highly statistically significant difference ( $p$  value  $< 0.001$ ) between VAP patients ( $11.37 \pm 2.53$  ng/ml) on one side and non VAP-ICU ( $5.07 \pm 3.50$  ng/ml) patients and healthy control subjects ( $4.12 \pm 2.9$  ng/ml) on the other side regarding the mean values of endocan. These results are in agreement with those of other investigators. Scherpereel et al. [13] observed that circulating endocan levels in the blood was related to the severity of sepsis and also reflected the outcome of the patient. De Freitas Caires et al. [12] have recently described elevated blood levels of cathepsin G-cleaved endocan in patients with sepsis. They

**Table 4** Comparison of some measurements between survivor and dead groups among VAP patients.

Outcome		APACHE II	SOFA	CPIS	Endocan 1st	Endocan 7th
Survivors (No = 22)	Mean $\pm$ SD	22.31 $\pm$ 3.73	6.40 $\pm$ 2.90	7.68 $\pm$ 1.04	10.35 $\pm$ 1.86	5.93 $\pm$ 1.56
Died (No = 20)	Mean $\pm$ SD	26.10 $\pm$ 4.66	9.50 $\pm$ 1.96	9.25 $\pm$ 2.02	12.50 $\pm$ 2.74	24.33 $\pm$ 7.14
<i>p</i> value		<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.05	<i>P</i> < 0.001

**Figure 2** Comparison of mean endocan levels between survivor and dead groups among VAP patients.

found that 20 out of 55 severe septic patients had increased plasma levels of endocan, whereas in the control subjects endocan was undetectable. In another study by Kao et al. [14] the plasma concentration of endocan, but not CRP or the WBC count, was correlated with the severity of pneumonia. Cox and his colleagues [15] measured plasma endocan, ICAM, and VCAM concentrations among 17 healthy male volunteers before and 4 h after the administration of 2 ng/kg lipopolysaccharide (LPS). They found that plasma levels of all measured cytokines, endocan, ICAM, and VCAM concentrations significantly increased after LPS administration. They suggested that endocan could be a novel marker of endothelial dysfunction in inflammatory conditions associated with endotoxemia. These results can be explained by the findings that sepsis and inflammation have associated with excess release of the inflammatory mediators like IL-1, TNF- $\alpha$  that induce endocan expression and blood levels of it may closely reflect the presence and severity of inflammation as well as the response to therapy [10].

Our study revealed the mean values of endocan were statistically significantly higher (*p* value < 0.001) among the dead VAP group than the survivor VAP group on both first day (12.50  $\pm$  2.74 ng/ml vs. 10.35  $\pm$  1.86 ng/ml respectively) and 7th day measurements (24.33  $\pm$  7.14 ng/ml vs. 5.93  $\pm$  1.56 ng/ml respectively). Also the mean values of endocan increased significantly (from 12.50  $\pm$  2.74 ng/ml to 24.33  $\pm$  7.14 ng/ml) among dead group while decreased significantly (from 12.50  $\pm$  2.74 ng/ml to 5.93  $\pm$  1.56 ng/ml) among the survivor group on repeated testing on the 7th day in comparison with the first day measurement. Other studies showed similar results. Palmiere et al. [16] investigated endocan, procalcitonin and C-reactive protein in postmortem serum from femoral blood in a series of sepsis-related fatalities and control individuals who underwent medicolegal investigations. Postmortem serum endocan concentrations were significantly higher in the sepsis group. Mihajlovic and his group [17] found the concentration of endocan was significantly higher in the

group of patients with sepsis induced organ failure, MODS development and in the group of non-survivors in contrast to group with less severe form of the disease, without multiorgan failure, and in contrast to a group of survivors (*p* < 0.05). Values of areas under the ROC curves showed that endocan levels had good discriminating power for a more severe course of sepsis, MODS development and possible discriminating power for mortality prediction (AUC: 0.81, 0.67, 0.71 retrospectively), better than PCT for fatality (AUC:0.53) and better than APACHE II (AUC:0.55) and SOFA (AUC:0.57) scores for organ failure. These results are explained intuitively by that the severe and non respondent pneumonia among dead patients is associated with high bacterial and toxin loads that stimulate release of the inflammatory mediators that induce excess release of endocan.

Our study found a high statistical positive correlation (*p* value < 0.001) between mortality prediction scores (APACHE II (*R* = 0.549), CRIP (*R* = 0.599) and SOFA (*R* = 0.517)) and endocan serum levels. These results are in agreement with those of other studies. A recent study reported that plasma endocan was superior to C-reactive protein and white blood cell count in evaluating the severity of pneumonia and had better correlation with pneumonia severity scores such as pneumonia severity index, CURB-65, and APACHE II scores [14]. Tang et al. [18] found that endocan levels significantly correlated with the APACHE II score in the ARDS patients (*R* = 0.676, *P* = 0.000). Endocan was significantly higher in nonsurvivors than in survivors (*P* = 0.017). Other investigators found similar results in different diseases [19–21].

## Conclusions

This study found increased endocan serum levels among VAP patients. We suggest a role for endocan in the diagnosis and prediction of the prognosis of VAP patients. We recommend other larger studies to augment our findings.

## Conflict of interest

We have no conflict of interest to declare.

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